

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF DR. YITZHAK PILPEL UNDER 37 C.F.R. § 1.132

I, Yitzhak Pilpel (Ph.D.), hereby declare as follows:

1. I am currently employed as a faculty member at the Department of Molecular Genetics of the Weizmann Institute, Israel. A true and correct copy of my *Curriculum Vitae* is attached to this declaration as Exhibit A. I am an author of [34 peer-reviewed scientific publications, of which 2 are related to microRNAs ("miRNAs"). I have supervised at least 3 experiments relating to microRNAs including computational mining of the mammalian microRNA repertoire, and experimental analyses of the regulatory agents controlling microRNAs.
2. A nucleic acid sequence of length 18-25 (endogenous or synthetic), which is predicted by a microRNA target algorithm, to bind to the 3' UTR of a mRNA in a manner characteristic of mirRNA:mRNA binding, when co-expressed with the target mRNA (such as in an in vitro reporter gene assay), is likely to inhibit expression of the protein encoded by that mRNA.
3. Binding of microRNA to mRNA is characterized by a) 5'-end 'seed' (full complementary between the first 1-8 base pairs of the 5' of a mir and its target), conserved, often flanked by adenosines; b) Compensatory 3'-end in cases in which there is insufficient base-pairing of the microRNA 5' seed; and c) Multiple binding sites on the same mRNA targets which are not obligatory but enhance the binding effect on target repression. The above characteristics were described by various publications, including: Lai and Starck (Nat Genet, 2002; PLoS Biol., 2003), Doench and Sharp (Genes Dev., 2004), Lai (Genome Biol., 2004), Vella et. al. (Chem. Biol., 2004) and Brennecke et. al. (PLoS Biol., 2005)
4. The effectiveness of microRNA targets prediction algorithms that are based on the above characteristics has been described by numerous studies. As an example, Lewis et. al. (Cell 2003), reported a microRNA target prediction algorithm, TargetScan, which is based on the above mentioned characteristics, and have biologically validated 11 out of 15 predicted targets tested, estimating the algorithm's false positive rate to be between 22 and 31 percent. Another algorithm, called miRanda (Enright et. al., Genome Biol. 2003), correctly recovered 9 out of 10 biologically validated targets, with an estimated 24-39% false positive rate.
5. **Table A** below provides a list of characteristic nucleic-acid-sequences:mRNA bindings, taken from Rosetta Genomics' patent applications. These nucleic acid sequences exhibit the characteristics of miRNA:mRNA bindings described above, and therefore are likely to inhibit their respective targets where co-expressed (such as in an in vitro reporter gene assay). Indeed, nearly all of the listed bindings are similarly detected by the above mentioned TargetScan algorithm, and half of them are further either detected by the miRanda algorithm, and/or are evolutionarily conserved.